No. 03-1237

IN THE

Supreme Court of the United States

MERCK KGAA,

Petitioner,

v.

INTEGRA LIFESCIENCES I, LTD. AND THE BURNHAM INSTITUTE,

Respondents.

ON WRIT OF CERTIORARI TO THE
UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

SUPPLEMENT JOINT APPENDIX

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CERTIORARI GRANTED JANUARY 7, 2005

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TABLE OF CONTENTS

SECTION 1: CHRONOLOGY OF EVENTS AND RELATIONSHIP TO FDA APPROVAL

Diagram: Time-line of Defendants' Scientific Inquiry and Drug Development Activities ..... SA 1

Diagram: The Five Stages of Merck's Drug Development Process ..... SA 2

Table: Uses of Data from Accused Experiments ... SA 3

Table: Testimony re Uses of Data from Accused Experiments ..... SA 6

FDA Form 1571: Investigational New Drug Application ..... SA 9

SECTION 2: PATENTS

Excerpts from Plaintiffs' U.S. Pat. No. 4,792,525, Dated December 20, 1998, and Asserted Claim No. 7 ..... SA 11

Excerpts from Plaintiffs' U.S. Pat. No. 4,789,734, Dated December 6, 1988, and Asserted Claim No. 1 ..... SA 13

Excerpts from Plaintiffs' U.S. Pat. No. 4,879,237, Dated November 7, 1989, and Asserted Claim No. 4 ..... SA 15

Excerpts from Plaintiffs' U.S. Pat. No. 5,695,997, Dated December 9, 1997, and Asserted Claims No. 15, 16, 17 & 18 ..... SA 18

Excerpts from Scripps' U.S. Pat. No. 5,573,230 Dated May 19, 1998, Describing and Claiming Dr. Cheresh's Invention using RGD peptides ... SA 20

SECTION 3: DOCUMENTS RELATING TO THE MERCK-SCRIPPS COLLABORATION

Excerpt from Research Grant Submitted January 31, 1995 by Dr. Friedlander ..... SA 22

Quarterly Report prepared for Merck's Business Area Team (BAT-ONC) Regarding Projected Development Plans for EMD-8, Dated September 1997 ..... SA 26

Merck Internal Document Discussing Plans for Clinical Development of EMD-8, Entitled "Project EMD 121974, International Development Plan (IDP)," Dated April 1, 1998 ..... SA 29

Merck Internal Memorandum from Lothar Finke, Head of Clinical Development to E. Schnurr, Dated February 3, 1997, and Translation ..... SA 39

SECTION 4: SCIENTIFIC ILLUSTRATIONS

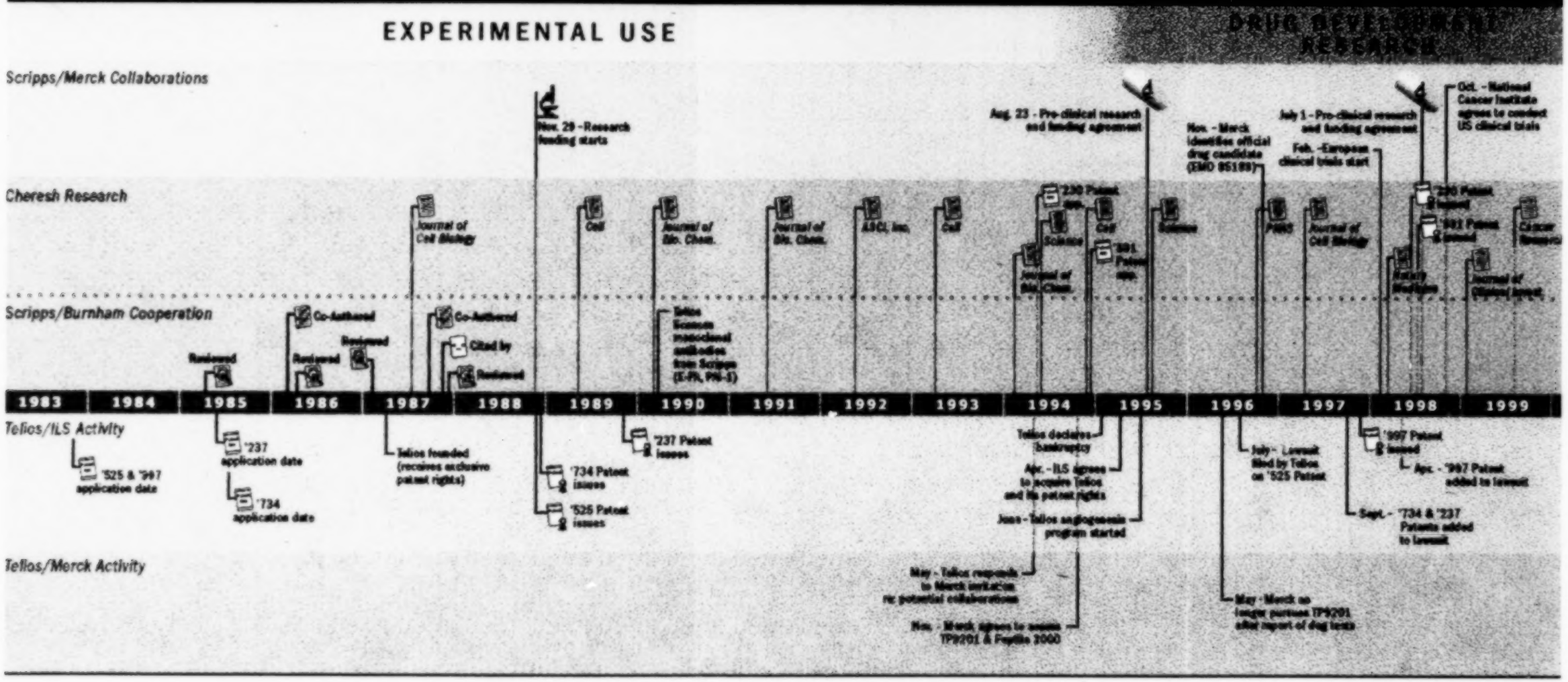
Chemical Structure of RGDS, a Linear Peptide Containing the RGD Binding Domain ..... SA 41

Chemical Structure of EMD-6 ..... SA 42

Chemical Structure of EMD-8 ..... SA 43

Chemical Structure of EMD-12 ..... SA 44

Defendants' Scientific Inquiry and Drug Development Activities



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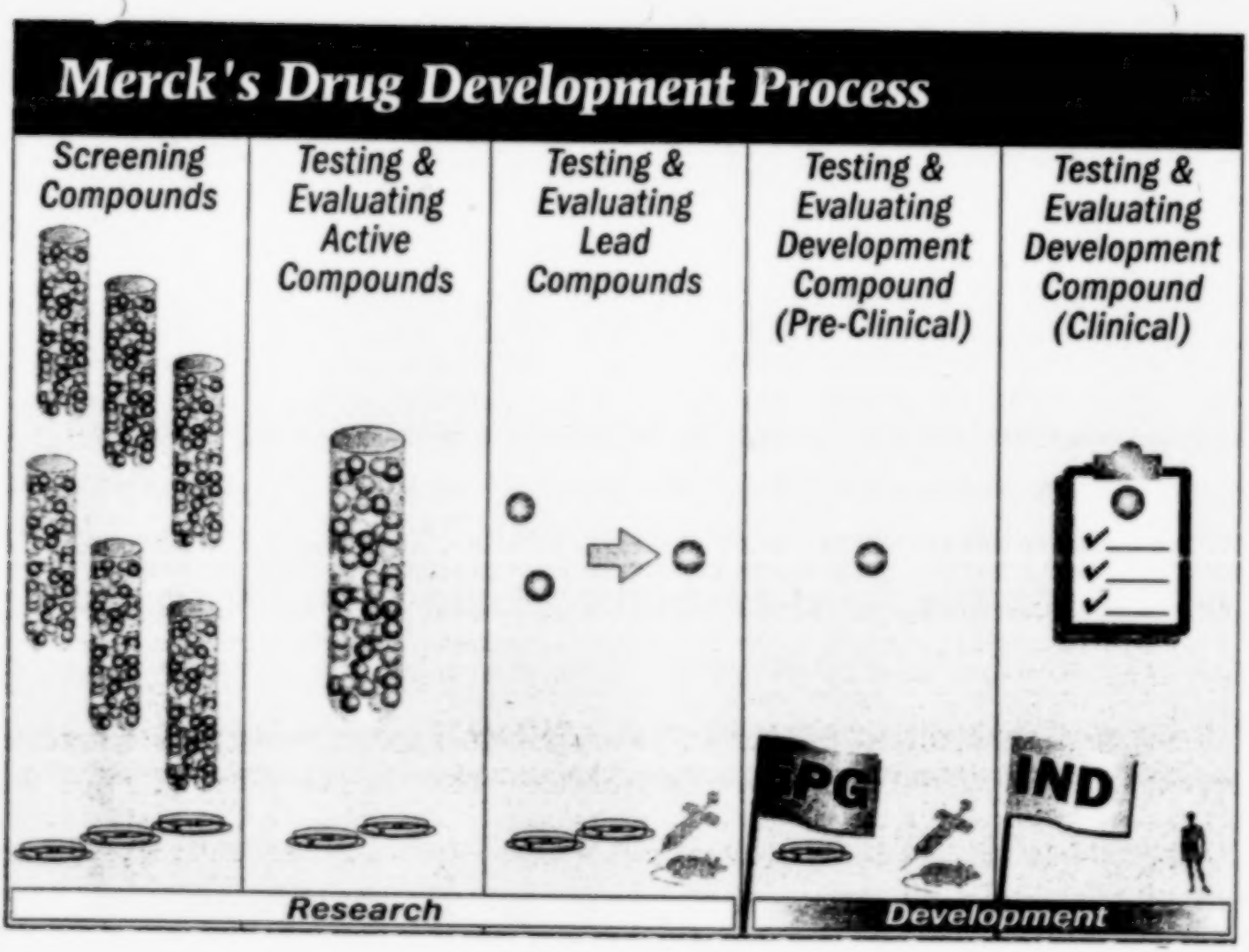
Merck's Drug Development Process
Screening Compounds
Testing & Evaluating Active Compounds
Testing & Evaluating Lead Compounds
Testing & Evaluating Development Compound (Pre-Clinical)
Testing & Evaluating Development Compound (Clinical)
IND
Research
Development

Uses of Data from Accused Experiments
(by category)

EXPERIMENT CATEGORY	SUMMARY OF DESCRIPTION PROVIDED BY DR. DEDHAR	NO. OF EXPS.	EXP. DATES	SAFETY	EFFICACY	MECHANISM OF ACTION	CANDIDATE SCREENING	PHARMACOLOGY (routes of administration and dosing)	PHARMACOKINETICS
Alpha (v)(B)(3)-Binding Assay	**inhibition of ligand adhesion:** coat plate with purified receptor (e.g. $\alpha_v\beta_3$); contact plate with radiolabeled or biotinylated ligand in the presence of test compound; rinse and observe extent of ligand adhesion	15	9/95-11/98		✓		✓		
Angiogenesis /Chick CAM Assay	**inhibition of angiogensis:** apply growth factor to 10-day CAM; subsequently, apply test compound; subsequently, observe number of blood vessel branch points	85	1/95-4/98	✓	✓	✓	✓		✓
Angio-Matrigel	**inhibition of angiogensis:** insert gel plug containing bFGF and test compounds subcutaneously in mouse; subsequently, recover and observe vascularization	2	1/98-2/98	✓	✓	✓	✓		
Cell Adhesion Assay	**inhibition of cell adhesion:** coat plate with protein; mix together and apply test compound and cells expressing $\alpha_v\beta_3$; rinse and observe amount of adhesion spectaphotometrically	25	7/95-7/98	✓	✓		✓		
Chemotaxis	**inhibition of cell migration:** allow PMN (polymorphonuclear) and PBMC (peripheral blood mocraphagic/moncytic) cells to migrate over fibronectin and collagen II toward chemo-attractant (synovial fluid); observe effect with and without test compound	1	1/97	✓		✓			
Chick-Embryo-Pharmaco-kinetics	**pharmacokinetics:** inject test substance in chick embryo; observe concentration of test compound in blood at intervals	1	8/94[1]						✓

SA3

[redacted]

[1]Though conducted pre-1995, this experiment was part of the drug development program

Uses of Data from Accused Experiments (by category)

EXPERIMENT CATEGORY	SUMMARY OF DESCRIPTION PROVIDED BY DR. DEDHAR	NO. OF EXPS.	EXP. DATES	SAFETY	EFFICACY	MECHANISM OF ACTION	CANDIDATE SCREENING	PHARMA-COLOGY (routes of administration and dosing)	PHARMACO-KINETICS
FACS	**inhibition of ligand adhesion:** fluorescent activated cell sorting machine used to assess the effect of test compound on the binding of penton base to $\alpha_v\beta_3$	1	7/98		✓	✓			
Mice Arthritis	**inhibition of angiogenesis:** inject mouse joints with ovaumin to induce arthritis; treat animal with test compound; observe degree of severity of arthritis	1	2/97		✓				
Mice Retina IF	**inhibition of angiogensis:** [Dr. Dedhar gives not details about these tests other than to say they were performed with EMD 80838, which Mr. Beckwith told him is fluorescein isothiocynate conjugated EMD 66203.]	3	3/96-5/96						
Mice-Retina-Vasculo	**inhibition of angiogensis:** treat newborn mice with test compound; subsequently, remove retinas and observe extent of vascularization	9	7/96-2/98						
Rabbit Arthritis	**inhibition of angiogensis:** inject rabbit joints with ovaumin to induce arthritis; treat animal with test compound; observe degree of severity of arthritis	5	2/97-10/97						
Rabbit Cornea Assay	**inhibition of angiogensis:** implant growth factor pellet in rabbit cornea; administer test compound; photograph eyes to observe neovascularization	14	9/95-10/98						
Rabbit Pharmaco-kinetics	**inhibition of angiogensis:** inject radioactively labeled test compound into rabbit; sample different tissues to determine where compound is found and its half-life	5	5/96-12/96						
Tumor Growth Chick CAM Assay	**inhibition of angiogenesis:** place M21-L melanoma cells (without $\alpha_v\beta_3$) on 10-day CAM; allow angiogenesis/tumor growth; apply test compound; subsequently, observe tumor weight	8	8/96-5/97		✓	✓	✓		

SA4

Uses of Data from Accused Experiments (by category)

EXPERIMENT CATEGORY	SUMMARY OF DESCRIPTION PROVIDED BY DR. DEDHAR	NO. OF EXPS.	EXP. DATES	SAFETY	EFFICACY	MECHANISM OF ACTION	CANDIDATE SCREENING	PHARMACOLOGY (routes of administration and dosing)	PHARMACOKINETICS
Tumor Growth in SCID-Mouse	**inhibition of angiogenesis:** implant human skin on immune-compromised mice; implant on skin non-$\alpha_v\beta_3$-containing melanoma cells; treat mouse with test compound; subsequently, observe size of tumors	2	6/96-8/96	✓	✓	✓		✓	
Tumor Growth/Nude Mice Assay	**inhibition of angiogenesis:** inject immune-compromised mice with non-$\alpha_v\beta_3$-containing melanoma cells; treat with test compound; subsequently, observe size of tumors	3	9/96-10/98		✓	✓		✓	✓

WD 031300/1/scb/1709885/v3

SA5

APPENDIX A

Testimony Regarding Uses of Data from Accused Experiments by Category

Experiment Category	No. of Exps.	Exp. Dates	Safety	Efficacy	Mechanism of Action	Candidate Screening	Pharmacology (routes of administration and dosing)	Pharmaco kinetics	Assay Included in IXSYS IND?
Alpha (v)(B)(3)-Binding Assay	15	9/95-11/98		Cheresh Tr. 2049:3-2050:2		Cheresh Tr. 2049:3-2050:2			
Angio-genesis/ Chick CAM Assay	85	1/95-4/98	Bynum Tr. 2325:1-19 Cheresh Tr. 2050:3-13	Cheresh Tr. 2050:3-2051:4	Cheresh Tr. 2050:3-2051:6	Cheresh Tr. 2050:3-2051:10		Cheresh Tr. 2050:3-2051:21	Cheresh Tr. 2042:7-19 Houston Tr. 3052:8-3053:9, 3053:17-23, 3059:23-3060:3, 3060:17-25
Angio-Matrigel	2	1/98-2/98	Cheresh Tr. 2051:22-2053:9	Cheresh Tr. 2051:22-2053:9	Cheresh Tr. 2051:22-2053:9	Cheresh Tr. 2051:22-2053:13			
Cell Adhesion Assay	25	7/95-7/98	Cheresh Tr. 2053:14-2054:17	Cheresh Tr. 2053:14-2054:17	Cheresh Tr. 2053:14-2054:18	Cheresh Tr. 2053:14-2054:20			

SA6

Testimony Regarding Uses of Data from Accused Experiments by Category

Experiment Category	No. of Exps	Exp. Dates	Safety	Efficacy	Mechanism of Action	Candidate Screening	Pharmacology (routes of administration and dosing)	Pharmaco kinetics	Assay Included in IXSYS IND?
Chemotaxis	1	1/97	Cheresh Tr. 2054:21-2056:14	Cheresh Tr. 2054:21-2056:14	Cheresh Tr. 2054:21-2056:2				
Click-Embryo-Pharmaco kinetics	1	8/94[1]						Cheresh Tr. 2056:15-2057:24	
FACS	1	7/98		Cheresh Tr. 2057:25-2058:15	Cheresh Tr. 2057:25-2058:15				
Mice arthritis	1	2/97		Cheresh Tr. 2058:17-2059:13					
Mice Retina IF	3	3/96-5/96						Friedlander Tr. 1870:17-1871:23	
Mice-Retina-Vasculo	9	7/96-2/98	Friedlander Tr. 1863:3-25	Friedlander Tr. 1831:24-1832:5; 1868:15-1869:13	Friedlander Tr. 1831:24-1832:13; 1833:1-1834:11; 1866:24-1868:14	Friedlander Tr. 1834:12-23, 1872:1-14	Friedlander Tr. 1869:14-1870:16	Friedlander Tr. 1831:24-1832:9	

SA7

[1] Though conducted pre-1995, this experiment was part of the drug development program.

Testimony Regarding Uses of Data from Accused Experiments by Category

Experiment Category	No of Exps.	Exp. Dates	Safety	Efficacy	Mechanism of Action	Candidate Screening	Pharmacology (routes of administration and dosing)	Pharmaco kinetics	Assay Included in IXSYS IND?
Rabbit Arthritis	5	2/97-10/97	Storgard Tr. 1596:17-1597:11, 1600:24-1601:15	Storgard Tr. 1597:12-1598:10	Storgard Tr. 1590:11-1593:10	Storgard Tr. 1593:11-1595:12	Storgard Tr. 1595:13-1596:16	Storgard Tr. 1595:13-1596:16	
Rabbit Cornea Assay	14	9/95-10/98	Friedlander Tr. 1863:3-25	Friedlander Tr. 1831:24-1832:4, 1863:3-1865:13, 1872:15-19	Friedlander Tr. 1831:24-1832:13, 1833:1-1834:11, 1849:21-1851:1	Friedlander Tr. 1834:12-1838:1			Cheresh Tr. 2047:7-2043:6 Houston Tr. 3053:10-16; 3059:23-3060:7; 3060:17-3061:10
Rabbit Pharmaco kinetics	5	5/96-12/96						Friedlander Tr. 1831:24-1832:9, 1865:14-1866:23	Cheresh Tr. 2047:7-2043:11
Tumor Growth Chick CAM Assay	8	8/96-5/97	Cheresh Tr. 2059:17-2060:8	Cheresh Tr. 2059:17-2060:8	Cheresh Tr. 2059:17-2060:7	Cheresh Tr. 2059:17-2060:10			
Tumor Growth in SCID-Mouse	2	6/96-8/96	Cheresh Tr. 2060:11-2062:19	Cheresh Tr. 2060:11-2062:19	Cheresh Tr. 2060:11-2062:19		Cheresh Tr. 2060:11-2062:14		Cheresh Tr. 2042:7-23 Houston Tr. 3053:17-23; 3059:23-3060:10; 3060:17-3061:12
Tumor Growth/Nude Mice Assay	3	9/96-10/98		Cheresh Tr. 2062:25-2063:16	Cheresh Tr. 2062:25-2063:17		Cheresh Tr. 2062:25-2063:16	Cheresh Tr. 2062:25-2063:17	Cheresh Tr. 2042:7-23

SA8

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

INVESTIGATIONAL NEW DRUG APPLICATION (IND)

(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) PART 312)

Form Approved: OMB No. 0910-0014.
Expiration Date: December 31, 1999
See OMB Statement on Reverse.

NOTE: No drug may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40).

1. NAME OF SPONSOR

2. DATE OF SUBMISSION

3. ADDRESS *(Number, Street, City, State and Zip Code)*

4. TELEPHONE NUMBER *(Include Area Code)*

5. NAME(S) OF DRUG *(Include all available names: Trade, Generic, Chemical, Code)*

6. IND NUMBER *(If previously assigned)*

7. INDICATION(S) *(Covered by this submission)*

8. PHASE(S) OF CLINICAL INVESTIGATION TO BE CONDUCTED: ☐ PHASE 1 ☐ PHASE 2 ☐ PHASE 3 ☐ OTHER _____ *(Specify)*

9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS (21 CFR Part 312), NEW DRUG OR ANTIBIOTIC APPLICATIONS (21 CFR Part 314), DRUG MASTER FILES (21 CFR Part 314.420), AND PRODUCT LICENSE APPLICATIONS (21 CFR Part 601) REFERRED TO IN THIS APPLICATION.

10. ***IND submission should be consecutively numbered. The initial IND should be numbered "Serial number: 000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.***

SERIAL NUMBER
— — —

11. THIS SUBMISSION CONTAINS THE FOLLOWING: *(Check all that apply)*

☐ INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND) ☐ RESPONSE TO CLINICAL HOLD

PROTOCOL AMENDMENT(S):
☐ NEW PROTOCOL
☐ CHANGE IN PROTOCOL
☐ NEW INVESTIGATOR

INFORMATION AMENDMENT(S):
☐ CHEMISTRY/MICROBIOLOGY
☐ PHARMACOLOGY/TOXICOLOGY
☐ CLINICAL

IND SAFETY REPORT(S):
☐ INITIAL WRITTEN REPORT
☐ FOLLOW-UP TO A WRITTEN REPORT

☐ RESPONSE TO FDA REQUEST FOR INFORMATION ☐ ANNUAL REPORT ☐ GENERAL CORRESPONDENCE

☐ REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED ☐ OTHER _____ *(Specify)*

CHECK ONLY IF APPLICABLE

JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO THE CITED CFR SECTION FOR FURTHER INFORMATION.

☐ TREATMENT IND 21 CFR 312.35(b) ☐ TREATMENT PROTOCOL 21 CFR 312.38(a) ☐ CHARGE REQUEST/NOTIFICATION 21 CFR312.7(d)

FOR FDA USE ONLY

CDR/OBAND/DGD RECEIPT STAMP

DDR RECEIPT STAMP

[redacted]

DIVISION ASSIGNMENT:

IND NUMBER ASSIGNED:

FORM FDA 1571 (1/97) PREVIOUS EDITION IS OBSOLETE PAGE 1 OF 2

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SA9

12. **CONTENTS OF APPLICATION**

This application contains the following items: *(Check all that apply)*

☐ 1. Form FDA 1571 *[21 CFR 312.23(a)(1)]*
☐ 2. Table of Contents *[21 CFR 312.23(a)(2)]*
☐ 3. Introductory statement *[21 CFR 312.23(a)(3)]*
☐ 4. General investigational plan *[21 CFR 312.23(a)(3)]*
☐ 5. Investigator's brochure *[21 CFR 312.23(a)(5)]*
☐ 6. Protocol(s) *[21 CFR 312.23(a)(6)]*
  ☐ a. Study protocol(s) *[21 CFR 312.23(a)(6)]*
  ☐ b. Investigator data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  ☐ c. Facilities data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
  ☐ d. Institutional Review Board data *[21 CFR 312.23(a)(6)(iii)(b)]* or completed Form(s) FDA 1572
☐ 7. Chemistry, manufacturing, and control data *[21 CFR 312.23(a)(7)]*
  ☐ Environmental assessment or claim for exclusion *[21 CFR 312.23(a)(7)(iv)(e)]*
☐ 8. Pharmacology and toxicology data *[21 CFR 312.23(a)(8)]*
☐ 9. Previous human experience *[21 CFR 312.23(a)(9)]*
☐ 10. Additional information *[21 CFR 312.23(a)(10)]*

13. IS ANY PART OF THE CLINICAL STUDY TO BE CONDUCTED BY A CONTRACT RESEARCH ORGANIZATION? ☐ YES ☐ NO

IF YES, WILL ANY SPONSOR OBLIGATIONS BE TRANSFERRED TO THE CONTRACT RESEARCH ORGANIZATION? ☐ YES ☐ NO

IF YES, ATTACH A STATEMENT CONTAINING THE NAME AND ADDRESS OF THE CONTRACT RESEARCH ORGANIZATION, IDENTIFICATION OF THE CLINICAL STUDY, AND A LISTING OF THE OBLIGATIONS TRANSFERRED.

14. NAME AND TITLE OF THE PERSON RESPONSIBLE FOR MONITORING THE CONDUCT AND PROGRESS OF THE CLINICAL INVESTIGATIONS

15. NAME(S) AND TITLE(S) OF THE PERSON(S) RESPONSIBLE FOR REVIEW AND EVALUATION OF INFORMATION RELEVANT TO THE SAFETY OF THE DRUG

I agree not to begin clinical investigations until 30 days after FDA's receipt of the IND unless I receive earlier notification by FDA that the studies may begin. I also agree not to begin or continue clinical investigations covered by the IND if those studies are placed on clinical hold. I agree that an Institutional Review Board (IRB) that complies with the requirements set fourth in 21 CFR Part 56 will be responsible for initial and continuing review and approval of each of the studies in the proposed clinical investigation. I agree to conduct the investigation in accordance with all other applicable regulatory requirements.

16. NAME OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE

17. SIGNATURE OF SPONSOR OR SPONSOR'S AUTHORIZED REPRESENTATIVE

18. ADDRESS *(Number, Street, City, State and Zip Code)*

19. TELEPHONE NUMBER *(Include Area Code)*

20. DATE

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FORM FDA 1571 (1/97) PAGE 2 OF 2

SA10

United States Patent [19]

Ruoslahti et al.

[11] **Patent Number: 4,792,525**

[45] **Date of Patent: *Dec. 20, 1988**

[54] **TETRAPEPTIDE**

[75] Inventors: **Erkki Ruoslahti**, Olivenhain; **Michael Pierschbacher**, San Diego, both of Calif.

[73] Assignee: **La Jolla Cancer Research Foundation**, La Jolla, Calif.

[*] Notice: The portion of the term of this patent subsequent to Mar. 25, 2003 has been disclaimed.

[21] Appl. No.: **744,981**

[22] Filed: **Jun. 17, 1985**

Related U.S. Application Data

[60] Division of Ser. No. 554,821, Nov. 22, 1983, Pat. No. 4,578,079, and a continuation-in-part of Ser. No. 405,239, Aug. 4, 1982, Pat. No. 4,517,686, and a continuation-in-part of Ser. No. 433,457, Oct. 8, 1982, abandoned, and a continuation-in-part of Ser. No. 518,036, Jul. 28, 1983, Pat. No. 4,589,881.

[51] **Int. Cl.**[4] **C12N 0/500**; C12N 11/08; C07K 7/10; C07K 17/02

[52] **U.S. Cl.** **435/240.243**; 435/180; 530/811; 530/330; 530/324; 530/812; 530/815

[58] **Field of Search** 435/240, 241, 244, 180; 530/815, 330, 353, 380

[56] **References Cited**

U.S. PATENT DOCUMENTS

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4,511,653 4/1985 Play et al. 435/69
4,578,079 3/1986 Ruoslahti et al. 435/178

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Lam et al., 1987, "Evidence that Argionyl-Glycyl-Aspartate Peptides and Fibrinogen & Chain Peptides Share a Common Binding Site on Platelets". Journal of Biological Chemistry 262: 947 at p. 947.
Babel et al., European Journal of Biochemistry, 143, pp. 545-556 (1984).

Primary Examiner—John E. Tarcza
Attorney, Agent, or Firm—Pretty, Schroeder, Brueggemann & Clark

[57] **ABSTRACT**

The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachent activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y, having said activity are disclosed.

11 Claims, 2 Drawing Sheets

CODE	SEQUENCE	CELL ATTACHMENT
FIBRONECTIN		0.10 emol/cm (• • • •)
IV	YAVTGRGDSPASSKPISINYRTEIDKPSQM(C)	0.26
IVA	VTGRGDSPASSKPI(C)	1.8
IVB	SINYRTEIDKPSQM(C)	> 50.0
IVA1	VTGRGDSPA(C)	2.5
IVA2	SPASSKPIS(C)	> 50.0
IVA12	VTGRGD(C)	15.0
IVA13	GRGDS(C)	3.0
IVA15	RGDSPA(C)	8.0
IVD5	RVDSPA(C)	> 50.0
TGRG	TGRG	—
RGDS	RGDS	• •
GDSP	GDSP	—

correlates with the presence of the cell attachment domain. One potential manifestation of the cell attachment activity of the synthetic peptides described here, and fragments thereof of like characteristics, is chemotactic activity.

Application of the ability of the invention to inhibit cell attachment when presented in a solubilized form may find utility in situations where it is desirable to prevent cell adhesion to a substrate or adhesion between cells. Undesirable cell attachment to various medical sutures, or dressings, may be prevented by presenting the invention in solubilized form. When the invention is used either in conjunction or combination with anothe molecule, such as a therapeutic agent, or particle treated with such an agent, the entrance of that agent or particle into the cell may be enhanced by the effect of the invention on the phagocytic activity of the cell, thereby increasing the efficiency of the therapeutic agent.

Although the invention has been described with regard to certain preferred embodiments, it should be understood that various changes and modifications, as would be obvious to one having the ordinary skill in this art, may be made without departing from the scope of the invention which is defined in the appended claims. For example, it may not be necessary to have the free acid at the C-terminus, as it may be amidated or substituted by some other group. Further, limited substitutions may be made to the basic tetrapeptide as illustrated by the substitution of Ser by Cys, without destroying the inherent cell attachment activity. Particular features of the invention are emphasized in the claims which follow.

Industrial Application

The invention is useful in surgery and therapeutic reconstruction and treatment of injuries.

What is claimed is:

1. A method for promoting cell attachment to a substrate comprising the steps of:

immobilizing on the substrate a non-naturally occurring polypeptide which includes as the cell-attachment-promoting constituent the sequence -Arg-Gly-Asp-R- wherein R is Ser, Cys, Thr or other amino acid such that the polypeptide has cell-attachment-promoting activity; and providing free cells for attachment to said substrate,.

2. A method for promoting cell attachment to a substrate comprising the steps of:

immobilizing on the substrate a composition of matter comprising a non-naturally occurring peptide

X-Arg-Gly-Asp-R-Y

wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Ser Thr, Cys or other amino acid, such that said peptide has cell-attachmentpromoting activity; and

providing free cells for attachment to the substrate.

3. A method of promoting cell attachment activity comprising the steps of:

immobilizing on a substrate a non-naturally occurring polypeptide including the sequence Arg-Gly-Asp-Ser; and

providing free cells for attachment to said substrate.

4. A method for promoting cell attachment to a substrate comprising the steps of:

immobilizing on the substrate a substantially pure peptide including as the cell-attachment-promoting constituent the amino acid sequence Arg-Gly-Asp-R wherein R is Ser, Cys, Thr or other amino acid, said peptide having cell-attachment-promoting activity, and said peptide not being a naturally occurring peptide; and

providing free cells for attachment to said substrate.

5. A method of promoting cell attachment to a substrate, comprising the steps of:

immobilizing on the substrate a polypeptide which includes as the cell-attachment-promoting constituent the amino acid sequence Arg-Gly-Asp-R wherein R is Ser, Cys, Thr or other amino acid such that the polypeptide has cell attachment promoting activity, said polypeptide having less than about 31 amino acid residues; and

providing free cells for attachment to said substrate.

6. A method of prommoting cell attachment to a substrate, comprising the steps of:

immobilizing on the substrate a composition of matter comprising a substantially pure polypeptide

X-Arg-Gly-Asp-R-Y

wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Ser, Syc, Thr or other amino acid, said peptide having cell attachment promoting activity, and said peptide not being a naturally occurring peptide; and

providing free cells for attachment to said substrate.

7. A method of promoting cell attachment to a substrate, comprising the steps of:

immobilizing on the substrate a composition of matter comprising a polypeptide

X-Arg-Gly-Asp-R-Y

wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Ser, Cys, Thr or other amino acid such that said peptide has cell attachment promoting activity, said polypeptide having less than about 31 amino acid residues; and

providing free cells for attachment to said substrate.

8. A substantially pure peptide including as the cell-attachment-promoting constituent the amino acid sequence Arg-Gly-Arg-R wherein R is Ser, Cys, Thr or other amino acid, said peptide having cell-attachment-promoting activity, and said peptide not being a naturally occurring peptide.

9. A composition of matter comprising the peptide of claim 8 attached to a substrate.

10. A peptide including as the cell-attachment-promoting constituent the amino acid sequence Arg-Gly-Asp-R, wherein R is Ser, Cys, Thr or other amino acid such that the peptide has cell-attachment-promoting activity, wherein said peptide has less than about 31 amino acid residues.

11. A composition of matter comprising the peptide of claim 10 attached to a substrate.

* * * * *

United States Patent [19]

Pierschbacher

[11] **Patent Number: 4,789,734**

[45] **Date of Patent: Dec. 6, 1988**

[54] **VITRONECTIN SPECIFIC CELL RECEPTOR DERIVED FROM MAMMALIAN MESENCHYMAL TISSUE**

[75] Inventor: Michael D. Pierschbacher, San Diego, Calif.

[73] Assignee: La Jolla Cancer Research Foundation, La Jolla, Calif.

[21] Appl. No.: 763,046

[22] Filed: Aug. 6, 1985

[51] Int. Cl.[4] C07K 15/14

[52] U.S. Cl. 530/395; 530/350; 530/413; 530/840; 530/842; 530/841; 530/380; 210/635

[58] Field of Search 530/395, 356

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Attorney, Agent, or Firm—Pretty, Schroeder Brueggemann & Clark

[57] **ABSTRACT**

A method of isolating cell surface receptors utilizing a short peptide sequence bound to an affinity column. Cell surface receptors which bind selectively to the short peptide and which are specific to various adhesion proteins may be isolated therewith from various cell preparations. These receptors, whose functional integrity has been maintained by the presence of the peptide ligand, are incorporated into liposomes and used to deliver specific compounds inside the liposomes to select tissues containing the specific adhesion proteins.

1 Claim, No Drawings

SA13

umn was further fractionated on a column of 1 ml wheat germ agglutinin-Sepharose ® (Pharmacia Fine Chemicals, Piscataway, N.J.), equilibrated with column buffer containing 0.1% NP-40 in the phosphate buffer saline detailed above. The receptor was eluted with 200 mM N-acetyl-glucosamine in the column buffer. The column fractions were analyzed by SDS-PAGE as described above, but the gell was stained with Coomassie blue.

EXAMPLE IV

Isolation of Platelet Receptor

Outdated human platelets (5 units) were harvested by centrifugation at 2200 rpm. The pellet was resuspended in Tyrode's buffer containing 1 mM $CaCl_2$, and platelets were again collected by centrifugation. Five ml of cold 10 mM phosphate, 150 mM $NaCl_2$, 1 mM $MgCl_2$, pH 7.3 (PBS), containing 50 mM octylthioglucoside (Behring Diagnostics, La Jolla, Calif.) and 3 mM PMSF were added to this pellet (5 ml). After resuspension of the pellet and incubation at 4° C. for 15 min., the lysate was centrifuged at 30,000 xg for 20 min. (4° C.), and the supernatant (5 ml) was applied to a GRGDSP-Sepharose ® column (bed volume 10 ml). the affinity matrix had been prepared by incubating 120 mg of GRGDSP(K)-peptide (the lysine residue (K) was added to optimize coupling) with 10 ml of CNBr-Sepharose ® following the manufacturer's instructions. The extract was incubated with the affinity matrix overnight at 4° C., and the column was washed with 50 ml of PBS containing 25 mM octylthioglucoside and 1 mM PMSF. Elution of specifically bound components was accomplished by washing the column with 10 ml of column buffer containing 1 mg/ml of GRGDSP-peptide, and then with another 10 ml of column buffer. Finally, the column was washed with 8 M urea, 50 mM tris-HCl, pH 7.5, to release non-specifically bound proteins. Fractions (2.5 ml) were collected after the peptide solution was applied. Sixty μl-aliquots of each fraction were mixed with 20 μl of 12% SDS, 20% 2-mercaptoethanol, 60 mM Tris-HCl, pH 6.8, boiled for 3 min., and electrophoresed on a 7.5% polyacrylamide gel according to the method of Laemmli, above. Protein bands were visualized by staining with Coomassie blue. The amount of protein recovered from the column was about 0.5 mg, as estimated by densitometric scanning of the gel bands.

EXAMPLE V

Incorporation of Vitronectin Receptor Into Liposomes

Phosphatidylcholine liposomes incorporating the cell surface receptors were prepared essentially by the method of Mimms et al., Biochemistry 20:883 (1981), which is incorporated by reference. Egg yolk phosphatidylcholine (Sigma, St. Louis, Mo.), ^{3}H-phosphatidycholine (New England Nuclear, Boston, Mass.) and the appropriate radiolodinated cell surface receptor fractions were dissolved in column buffer. This solution was then dialyzed against a detergent-free buffer, PBS for 24 hours at 4° C., resulting in the formation of liposomes with an average diameter of approximately 200 nm, as judged by electron microscopy. In the case of the 115 and 125 kD polypeptides of the vitronectin receptor, about 90% of the protein radioactivity was found associated with the liposome fraction after the liposomes had been floated to the surface of a sucrose gradient by ultracentrifugation.

EXAMPLE VI

Binding of Vitronectin Receptor-Liposomes to Vitronectin and Peptide Substrates

Liposomes incorporating the radioiodine labelled vitronectin receptor polypeptides were used to study the recognition specificity of the receptor. Polystyrene Microtiter plate wells (Linbro/Titertek, Inglewood, Calif.) were coated with extracellular matrix proteins and peptides including vitronectin, fibronectin, laminin and the synthetic peptides Gly-Arg-Gly-Asp-Ser-Pro (GRGDSP) and Gly-Arg-Gly-Glu-Ser-Pro (GRGESP). The vitronectin receptor-liposome preparation showed dose dependent binding to microtiter wells coated with vitronectin, but not fibronectin or laminin. Moreover, it bound to the GRGDSP peptide which contains the Arg-Gly-Asp sequence, but not the GRGESP peptide, which does not. The Arg-Gly-Asp sequence is therefore implicated in the binding of the liposomes containing the vitronectin receptor.

EXAMPLE VII

Use of the Receptor-Liposome Preparation to Target Therapeutic Agents

Receptor liposomes are prepared as described above and a desired therapeutic agent is incorporated into them according to published methods (Hashimoto et al., Cancer Research 43:5328 (1983); Gregoriadis and Senior, Biochem. Soc. Trans. 12:337 (1984)) which are incorporated by reference. These conjugated liposomes are injected intravenously using 1-300 μmol of lipid per kg of body weight or applied locally. Such vitronectin receptor liposomes are used, for example, to target tissue degrading enzymes to tumors, which contain large amounts of connective tissue. Moreover, platelet receptor conjugated liposomes are used, for example, to deliver clot dissolving agents specifically to blood clots in the body, such as those causing a myocardial infarction.

EXAMPLE VIII

Use of the Receptor as a Coating on a Substrate

To coat a nonlipid surface with receptors, a receptor from a solution is covalently coupled to a surface such as plastic using one of the many well known methods available for such coupling. Receptor fragments that retain the ligand-binding activity but lack the membrane embedded portion of the molecule are advantageously used as they are more soluble than the complete receptor. Such materials coated with receptors are useful as prostheses where attachment of extracellular matrix is desired, such as, for example, artificial skin.

We claim:

1. A substantially purified cell surface receptor derived from mesenchymal tissue and capable of binding to a peptide containing the amino acid sequence Arg-Gly-Asp, comprising a glycoprotein composed of at least two polypeptides of about 115 and 125 kD, respectively, as determined by SDS-PAGE under reducing conditions which selectively binds to vitronectin, but not to fibronectin.

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SA14

United States Patent [19]

Rudslahti et al.

[11] **Patent Number: 4,879,237**

[45] **Date of Patent: Nov. 7, 1989**

[54] **USE OF PEPTIDES IN CONTROL OF CELL ATTACHMENT AND DETACHMENT**

[75] Inventors: **Eric I. Ruoslahti**, Rancho Santa Fe; **Edward G. Hayman**, Oceanside; **Michael D. Pierschbacher**, San Diego, all of Calif.

[73] Assignee: **La Jolla Cancer Research Foundation**, La Jolla, Calif.

[21] Appl. No.: **738,078**

[22] Filed: **May 24, 1985**

[51] Int. Cl.[4] C12N 5/00

[52] U.S. Cl. 435/240.2; 435/240.21; 530/331

[58] Field of Search 435/68, 240.2, 240.25, 435/240.21; 530/329, 330, 331

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Attorney, Agent, or Firm—Pretty, Schroeder, Brueggemann & Clark

[57] **ABSTRACT**

A method of using synthetic cell attachment-promoting peptides from fibronectin to detach cultured cells from the substratum is described.

9 Claims, 8 Drawing Sheets

Discussion

The significance of the present invention is far reaching and of considerable importance in research, diagnostics, and in cell biology generally.

Consider, for example, the value of the method of this invention in the production of cell lines and products of cell lines. The ability to grow cells on substrata and then to remove the cells gently and nontoxically from the substrate opens new avenues of cell production. Some cell lines will propagate only when attached to substrates and others propagate much more efficiently when attached to substrates. Attachment to semipermeable membranes, for example, followed by detachment in accordance with this invention will permit selected nutrients to be provided through the membrane. The production of cell lines for producing a multitude of products, may be greatly simplified by use of the present invention. In some instances, it is advantageous to keep cells detached. An example is the propagation of hybridoma cells producing monoclonal antibodies in a fermentation. The present invention, accordingly, has many and varied values and applications.

The diversity of cell types and culture conditions in which the polypeptides consisting essentially of ARG-GLY-ASP detach cells demonstrate a mechanism which is intrinsic to cells, and is not dependent upon toxicity or secondary mechanism. In vivo treatments using the present invention is, therefore, quite feasible, according to known principles. For example, Boucaut, et al. (1984) have shown that the ARG-GLY-ASP characterized peptide prevented gastrulation in amphibian embryos. In vivo applications of the present invention are considered to include mobilization of bone marrow cells (see Patel, et al., Proc. Nat'l Acad. Sci. U.S.A. (1984) 82, 440-444), prevention and reversal of attachment of disseminated tumor cells locally, such as in the case of an operation performed in the peritoneal cavity, and prevent of adhesions and scar formations locally, as, for example, in the case of eye operations. Since *E.Coli* have a surface protein which includes an ARG-GLY-ASP sequence (Pierschbacher & Ruoslahti, 1984c) it may be possible to accomplish prophylactic inhibition of *E.coli* binding to epithelial cells of the urinary tract or intestine may be accomplished through the principles of this invention, thus preventing or reducing the risk of infection. Diagnosis of *E.coli* related infections and the treatment of existing infections by the detachment of *E.coli* may be accomplished within the scope of the present invention. Identification of various pathogenic bacteria strains is also within the scope of the inventive principle of the present invention.

Cell attachment may be controlled as to time, rate, location or amount. For example, it is sometimes desirable to permit cell attachment in some areas before permitting attachment in others. Since the method of this invention is non-toxic and totally reversible, cell attachment may be prevented by bathing a given area with the active polypeptide of this invention during a given period of time and thereafter washing the area free of the polypeptide. Cyclic processes in which cells are permitted to attach, and then are detached and then permitted to attach, in a cyclic stepwise or continuous manner become quite feasible. For example, a continuous belt or ribbon may be carried through a cell production vat, through a cell detachment vat where the cells are harvested, through washing or other treating stations and then back to the cell production vat. Fermentation processes may be greatly accelerated by providing large surface area substrates from which cells are easily harvested using the present invention. The important applications of the present invention are so broad and diversified as almost to defy cataloging at this time.

It is contemplated, based upon present information and techniques, that the polypeptides of this invention will generally be synthetic and can be produced by the Merrifield method, or modifications thereof. Polypeptides which consist essentially of the ARG-GLY-ASP amino acid sequence, as described above, may also be produced by enzyme cleavage and by isolation techniques and, thus produced, would, if sufficiently pure, be considered to be synthetic for purposes of the present invention though perhaps not in the classic definition of synthetic. Typically, however, the better approach is to fully synthesize the polypeptides from the amino acids.

The effective concentration used in the method of this invention is very broad. For example, a few micrograms per milliliter will control the rate of cell attachment in some environments, whereas from 1 to 100 milligrams per ml. may be necessary to detach cells from some substrates. As a general rule, however, effective concentrations will range from 0.1 micrograms per ml. to 100 mg./ml and preferably from 0.1 mg./ml. to 10 mg./ml. of carrier solution. The carrier solution is noncritical. The only requirements being that it be nontoxic to the cells and tissues and that it not contain seriously competing or interfering species. Typical buffer and nutrient solutions are quite satisfactory, as demonstrated hereinbefore.

Industrial Application

This invention finds application in the production of cell lines for research, in diagnosis and therapy, and in the industrial production of cell biological products.

What is claimed is:

1. A method of controlling the Arg-Gly-Asp mediated attachment of animal cells to substrates comprising the steps of:

(a) contacting a substrate normally capable of accepting cells with cells normally capable of attaching to such substrate; and

(b) contacting the substrate with a solution containing a polypeptide consisting essentially of the sequence GLY-ARG-GLY-ASP-ALA-PRO.

2. A method of preventing the Arg-Gly-Asp mediated attachment of animal cells to a substrate comprising contacting the cells with a polypeptide consisting essentially of the sequence GLY-ARG-GLY-ASP-ALA-PRO either before contacting the substrate with cells capable of attaching to the substrate or simultaneously with contacting such cells to such substrate.

3. A method of producing animal cells comprising the steps of:

(a) growing the cells on a substrate;

(b) contacting the cells with a solution containing a polypeptide consisting essentially of the sequence GLY-ARG-GLY-ASP- ALA-PRO; and

(c) harvesting cells detached from the substrate.

4. A method for detaching animal cells from a substrate to which they are bound in an Arg-Gly-Asp mediated manner, comprising contacting said bound cells with a solution containing a non-naturally occurring peptide consisting essentially of the amino acid sequence Arg-Gly-Asp-Y, is any amino acid such that the peptide has cell-detachment activity.

5. The method of claim 4 wherein Y further comprises a Ser, Thr, Cys, or Ala residue adjacent said Asp residue.

6. A method for harvesting animal cells grown on a substrate to which said cells attach in an Arg-Gly-Asp mediated manner comprising the steps of:

contacting said bound cells with a solution containing a non-naturally occurring peptide consisting essentially of the amino acid sequence Arg-Gly-Asp-Y, wherein Y is any amino acid such that the peptide has cell detachment promoting activity; and

harvesting cells detached from said substrate.

7. The method of claim 6 wherein Y further comprises a Ser, Thr, Cyc or Ala residue adjacent said Asp residue.

8. A method of detaching animal cells from a substrate to which they are bound in an Arg-Gly-Asp mediated manner, comprising contacting said bound cells with a peptide consisting essentially of the amino acid sequence X-Arg-Gly-Asp-Y wherein X is zero to thirty amino acids and Y is one to thirty amino acids, such that the peptide has cell detachment promoting activity.

9. A method for harvesting animal cells grown on a substrate to which said cells attach in an Arg-Gly-Asp mediated manner, comprising the steps of:

contracting said bound cells with a solution containing a peptide consisting essentially of the amino acid sequence X-Arg-Gly-Asp-Y wherein X is zero to thirty amino acids and Y is one to thirty amino acids, such that the peptide has cell detachment promoting activity; and

harvesting cells detached from said substrate.

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SA17

US005695997A

United States Patent [19]

Ruoslahti et al.

[11] Patent Number: 5,695,997

[45] Date of Patent: *Dec. 9, 1997

[54] **TETRAPEPTIDE**

[75] Inventors: **Erkki I. Ruoslahti**, Rancho Santa Fe; **Michael D. Pierschbacher**, San Diego, both of Calif.

[73] Assignee: **La Jolla Cancer Research Foundation**, La Jolla, Calif.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. Nos. 4,792,525 and 4,879,237 and 4,578,079.

[21] Appl. No.: **459,641**

[22] Filed: **Jun. 2, 1995**

Related U.S. Application Data

[63] Continuation of Ser. No. 902,472, Jun. 19, 1992, which is a continuation of Ser. No. 660,526, Feb. 25, 1991, abandoned, which is a continuation of Ser. No. 242,713, Sep. 9, 1988, Pat. No. 5,041,380, which is a division of Ser. No. 744,981, Jun. 17, 1985, Pat. No. 4,792,525, which is a division of Ser. No. 554,821, Nov. 22, 1993, Pat. No. 4,578,079, which is a continuation-in-part of Ser. No. 405,239, Aug. 4, 1982, Pat. No. 4,517,686, and a continuation-in-part of Ser. No. 433,457, Oct. 8, 1982, abandoned, and a continuation-in-part of Ser. No. 518,036, Jul. 28, 1983, Pat. No. 4,589,881.

[51] **Int. Cl.**[6] **C12N 5/02**; C07K 5/10; C07K 4/00; C07K 14/00

[52] **U.S. Cl.** **435/375**; 435/378; 435/402; 530/330

[58] **Field of Search** 530/329, 330, 530/331; 514/17, 18; 435/240.1, 240.2, 325, 375, 378, 402, 7.1

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[57] **ABSTRACT**

The peptide X-Arg-Gly-Asp-R-Y wherein X is H or at least one amino acid and Y is OH or at least one amino acid, and R is an amino acid selected from Thr or Cys, or other amino acid, having the same cell-attachment activity as fibronectin and the peptide X-Arg-Gly-Asp-Ser-Y, wherein X and Y having said activity are disclosed.

18 Claims, 3 Drawing Sheets

SA18

agent, or particle treated with such an agent, the entrance of that agent or particle into the cell may be enhanced by the effect of the invention on the phagocytic activity of the cell, thereby increasing the efficiency of the therapeutic agent.

Although the invention has been described with regard to certain preferred embodiments, it should be understood that various changes and modifications, as would be obvious to one having the ordinary skill in this art, may be made without departing from the scope of the invention which is defined in the appended claims. For example, it may not be necessary to have the free acid at the C-terminus, as it may be amidated or substituted by some other group. Further, limited substitutions may be made to the basic tetrapeptide as illustrated by the substitution of Set by Cys, without destroying the inherent cell attachment activity. Particular features of the invention are emphasized in the claims which follow.

INDUSTRIAL APPLICATION

The invention is useful in surgery and therapeutic reconstruction and treatment of injuries.

What is claimed is:

1. A method of altering cell attachment activity of cells, comprising:

contacting the cells with a substantially pure soluble peptide including RGDX where X is an amino acid and the peptide has cell attachment activity.

2. The method of claim 1 wherein said cell attachment activity is increased.

3. The method of claim 1 wherein said cell attachment activity is decreased.

4. A method of altering cell attachment activity of cells, comprising:

contacting the cells with a non-naturally occurring peptide including RGDX where X is an amino acid and the peptide has cell attachment activity.

5. The method of claim 4 wherein said peptide is in soluble form.

6. The method of claim 4 wherein said peptide is immobilized on a surface.

7. The method of claim 4 wherein said cell attachment activity is increased.

8. The method of claim 4 wherein said cell attachment activity is decreased.

9. A method of altering cell attachment activity of cells, comprising:

contacting the cells with a peptide including RGDX where X is an amino acid and the peptide has cell attachment activity and the peptide has less than about 31 amino acids.

10. The method of claim 9 wherein said peptide is in soluble form.

11. The method of claim 9 wherein said peptide is immobilized on a surface.

12. The method of claim 9 wherein said cell attachment activity is increased.

13. The method of claim 9 wherein said cell attachment activity is decreased.

14. A method of blocking cell surface receptors which mediate cell attachment activity, comprising:

contacting said cell surface receptors with a substantially pure soluble peptide including RGDX where X is an amino acid and the peptide has cell attachment activity.

15. A method of blocking cell surface receptors which mediate cell attachment activity, comprising:

contacting said cell surface receptors with a non-naturally occurring peptide including RGDX where X is an amino acid and the peptide has cell attachment activity.

16. The method of claim 15 wherein said peptide is in soluble form.

17. A method of blocking cell surface receptors which mediate cell attachment activity, comprising:

contacting said cell surface receptors with a peptide including RGDX where X is an amino acid and the peptide has cell attachment activity and the peptide has less than about 31 amino acids.

18. The method of claim 17 wherein said peptide is in soluble form.

* * * * *

US005753230A

United States Patent [19]

Brooks et al.

[11] Patent Number: 5,753,230

[45] Date of Patent: May 19, 1998

[54] **METHODS AND COMPOSITIONS USEFUL FOR INHIBITION OF ANGIOGENESIS**

[75] Inventors: **Peter Brooks**, San Diego; **David A. Cheresh**, Cardiff, both of Calif.

[73] Assignee: **The Scripps Research Institute**, La Jolla, Calif.

[21] Appl. No.: **210,715**

[22] Filed: **Mar. 18, 1994**

[51] **Int. Cl.**[6] **A61K 39/395**; A61K 49/00; C87K 16/28; C07K 16/18

[52] **U.S. Cl.** **424/158.1**; 424/143.1; 424/145.1; 424/155.1; 424/174.1; 530/388.24; 530/388.8; 530/388.25; 530/388.22

[58] **Field of Search** 424/143.1, 145.1, 424/158.1, 155.1, 174.1; 530/388.24, 388.8, 388.25, 388.22

[56] **References Cited**

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Primary Examiner—Susan A. Loring
Attorney, Agent, or Firm—Thomas Pitting; Emily Holmes

[57] **ABSTRACT**

The present invention describes methods for inhibition of angiogenesis in tissues using vitronectin $\alpha_v\beta_3$ antagonists, and particularly for inhibiting angiogenesis in inflamed tissues and in tumor tissues and metastases using therapeutic compositions containing anti-$\alpha_v\beta_3$ monoclonal antibodies.

41 Claims, 14 Drawing Sheets

BRIEF DESCRIPTION OF THE INVENTION

The present invention disclosure demonstrates that angiogenesis in tissues requires integrin $\alpha_v\beta_3$, and that inhibitors of $\alpha_v\beta_3$ can inhibit angiogenesis. The disclosure also demonstrates that antagonists of other integrins, such as $\alpha_v\beta_5$, or $\alpha_5\beta_1$, do not inhibit angiogenesis, presumably because these other integrins are not essential for angiogenesis to occur.

The invention therefore describes methods for inhibiting angiogenesis in a tissue comprising administering to the tissue a composition comprising an angiogenesis-inhibiting amount of an $\alpha_v\beta_3$ antagonist.

The tissue to be treated can be any tissue in which inhibition of angiogenesis is desirable, such as diseased tissue where neo-vascularization is occurring. Exemplary tissues include inflamed tissue, solid tumors, metastases, and the like tissues.

An $\alpha_v\beta_3$ antagonist for use in the present methods is capable of binding to $\alpha_v\beta_3$ and competitively inhibiting the ability of $\alpha_v\beta_3$ to bind to a natural ligand. Preferably, the antagonist exhibits specificity for $\alpha_v\beta_3$ over other integrins. In a particularly preferred embodiment, the $\alpha_v\beta_3$ antagonist inhibits binding of fibrinogen or other RGD-containing ligands to $\alpha_v\beta_3$ but does not substantially inhibit binding of fibrinogen to $\alpha_{IIb}\beta_3$. A preferred $\alpha_v\beta_3$ antagonist can be a polypeptide or a monoclonal antibody, or functional fragment thereof, that immunoreacts with $\alpha_v\beta_3$.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings forming a portion of this disclosure:

FIGS. 1A–1D illustrate the tissue distribution of the integrin subunits, β_3 and β_1, in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to β_3 and β_1 was performed as described in Example 3A. FIGS. 1A and 1B respectively illustrate the immunoreactivity of anti-β_3 in normal skin and granulation tissue. FIGS. 1C and 1D respectively illustrate the immunoreactivity of anti-β_1 in normal skin and granulation tissue.

FIGS. 2A–2D illustrate the tissue distribution of the von Willebrand factor and laminin ligands that respectively bind the β_3 and β_1 integrin subunits in normal skin and in skin undergoing wound healing designated as granulation tissue. Immunohistochemistry with antibodies to von Willebrand factor (anti-vWF) and laminin (anti-laminin) was performed as described in Example 3B. FIGS. 2A and 2B respectively illustrate the immunoreactivity of anti-vWF in normal skin and granulation tissue. FIGS. 2C and 2D respectively illustrate the immunoreactivity of anti-laminin in normal skin and granulation tissue.

FIGS. 3A–3D illustrate the tissue distribution of the vitronectin integrin receptor, $\alpha_v\beta_3$, tissue biopsies of bladder cancer, colon cancer, breast cancer and lung cancer, respectively. Immunohistochemistry with the LM609 antibody against $\alpha_v\beta_3$ was performed as described in Example 3C.

FIG. 4 illustrates a typical photomicrograph of a CAM of this invention devoid of blood vessels in an untreated 10 day old chick embryo. The preparation is described in Example 5B.

FIGS. 5A–5C illustrate the tissue distribution of the integrins β_1 and $\alpha_v\beta_3$ in the CAM preparation of this invention. FIG. 5A shows the distribution of the β_1 subunit in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with CSAT, an anti-β_1 antibody. FIG. 5B shows the distribution of the $\alpha_v\beta_3$ receptor in an untreated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an anti-$\alpha_v\beta_3$ antibody. FIG. 5C shows the distribution of the $\alpha_v\beta_3$ receptor in an βFGF treated 10 day old CAM preparation as detected by immunofluorescence immunoreactivity with LM609, an anti-$\alpha_v\beta_3$ antibody. The treatments and results are described in Example 5C.

FIG. 6 illustrates the quantification in a bar graph of the relative expression of $\alpha_v\beta_3$ and β_1 in untreated and βFGF treated 10 day old CAMs as described in Example 6A. The mean fluorescence intensity is plotted on the Y-axis with the integrin profiles plotted on the X-axis.

FIGS. 7A–7C illustrates the appearance of an untreated 10 day old CAM, a βFGF treated CAM, and a TNFα treated CAM, respectively, the procedures and results of which are described in Example 6A.

FIGS. 8A–8E illustrate the effect of topical antibody treatment on FGF-induced angiogenesis in a day 10 CAM as described in Example 7A1). FIG. 8A shows an untreated CAM preparation that is devoid of blood vessels. FIG. 8B shows the infiltration of new vasculature into an area previously devoid of vasculature induced by βFGF treatment. FIGS. 8C, 8D and 8E respectively show the effects of antibodies against β_1 (anti-β_1; CSAT), $\alpha_v\beta_3$ (anti-$\alpha_v\beta$ %; P3G2) and $\alpha_v\beta_3$ (anti-$\alpha_v\beta_3$; LM609).

FIGS. 9A–9C illustrate the effect of intravenous injection of synthetic peptide 66203 on angiogenesis induced by tumors as described in Example 7D2). FIG. 9A shows the lack of inhibitory effect of intravenous treatment with a control peptide (control peptide tumor) on angiogenesis resulting from tumor induction. The inhibition of such angiogenesis by intravenous injection of peptide 66203 (cyclic RGD tumor) is shown in FIG. 9B. The lack of inhibitory effects or cytotoxicity on *mature* preexisting vessels following intravenous infusion of peptide 66203 in an are a adjacent to the tumor-treated area is shown in FIG. 9C (cyclic RGD adjacent CAM).

FIGS. 10A–10C illustrate the effect of intravenous application of monoclonal antibodies to growth factor induced angiogenesis as described in Example 7B$_1$). FIG. 10A shows βFGF-induced angiogenesis not exposed to antibody treatment (control). No inhibition of angiogenesis resulted when a similar preparation was treated with anti-$\alpha_v\beta_3$ antibody P3G2 as shown in FIG. 10B. Inhibition of angiogenesis resulted with treatment of anti-$\alpha_v\beta_3$ antibody LM609 as shown in FIG. 10C.

FIGS. 11A and 11C illustrate the effect on embryonic angiogenesis following topical application of anti-integrin antibodies as described in Example 7C. Angiogenesis was not inhibited by treatment of a 6 day CAM with anti-β_1 and anti-$\alpha_v\beta_3$ antibodies respectively shown in FIGS. 11A and 11B. In contrast, treatment with the anti-$\alpha_v\beta_3$ antibody LM609 resulted in the inhibition of blood vessel formation as shown in FIG. 11C.

FIG. 12 illustrates the quantification of the number of vessels entering a tumor in a CAM preparation as described in Example 7D1). The graph shows the number of vessels as plotted on the Y-axis resulting from topical application of either CSAT (anti-β_1), LM609 (anti-$\alpha_v\beta_3$) or P3G2 (anti-$\alpha_v\beta_3$).

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide

LIONS SIGHTFIRST DIABETIC RETINOPATHY RESEARCH PROGRAM
AMERICAN DIABETES ASSOCIATION
National Center
1660 Duke Street
Alexandria, VA 22314

Please indicate type of award you are applying for:

_X_ LCIF Clinical Research Grant
___ LCIF Training Grant
___ LCIF Equipment Grant

PAGES 1-6 TO BE COMPLETED BY ALL APPLICANTS

1. TITLE OF PROPOSAL: Inhibition of ocular angiogenesis by antagonists of integrin

2. PRINCIPAL INVESTIGATOR: Martin Friedlander DEGREE(S): M.D

   POSITION: Assistant Member

   INSTITUTION NAME: The Scripps Research Institute

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4. CITIZENSHIP: US

5. Name, title, address, telephone number and signature of responsible financial officer to whom funds sho be sent, and who will keep a full account of disbursements:

   (print)
   Name: Kaye I. Wynne Signature: Kaye I. Wynne

   Title: Director, Grants & Contracts Address: The Scripps Research Institute

   Phone Number: 619/554-4999 10666 North Torrey Pines Road

   La Jolla, CA 92037

Friedlander EXHIBIT NO. 1 KARLA M. BAEZ

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8. Is support for this project being sought elsewhere? Yes X No ___

If "yes", from what agencies? List below the titles of projects, total funding requested, specify areas w there are overlaps in budget requests and indicate the expected starting date for funding. (If suppo obtained from other sources, funds awarded by the American Diabetes Association will be withdrawn)

NIH; There are no budgetary overlaps. The money being requested from ADA is to support the transition from preclinical to clinical trials. In order to proceed with an IND application for phase I clinical trials, we need to repeat many of ou animal studies with Good Laboratory Procedure (GLP) protocols. Toxicology, bio-availability and pharmacokinetic studies also need to be completed. The requeste funds are for a GLP qualified animal technician, equipment for bioassays and a sm supply budget. None of these are in our NIH grant.

9. Is this project or related projects currently being supported from other sources? Yes___ No X

If "yes", from what source(s) and for how long? List below the titles of projects, role of the applican each grant, percent of time devoted to each grant, and amount of total funding per year.

10. List all sources of support (pending or current) including federal (NIH, VA, NSF, etc.), foundatic industrial or other. Give complete title of all grants as well as total award, yearly award, inclusive fund dates, and the role of the applicant, and percent of time devoted to each grant. ATTACH THE ABSTRA PAGE OF ALL SOURCES OF SUPPORT (PENDING OR CURRENT). Ambiguity in the matter of ot funding will result in administrative disapproval of the application.

None. PI's lab is supported solely by institutional start up funds.

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Budget Justification

Technical personnel. One half time animal health technician, qualified for Good Laboratory Practice (GLP) and Quality Assurance (QA), is requested to supervise and participate in assuring that the animal studies are done according to GLP standards. This is essential if the preclinical studies are to be useful for translating the use of this reagent into a therapeutic in clinical trials. Currently, no GLP qualified animal technician is available at Scripps.

Equipment. Once again, in order to apply for an IND and clinical trials, a large amount of preclinical information is needed, including pharmacokinetics, bioavailability and toxicology. Since one of the reagents we are testing is an antibody, immunoassays (colorimetric-based ELISA assays) will be used to measure concentrations and distribution of the antibody. This will require the use of a microplate reader. We do not have such a piece of equipment in our laboratory. Since there will be a substantial increase in the number of animals studied, we will be generating large numbers of photographs to be analyzed. Our current hard drive storage space is limited, and it will be important to maintain permanent records of the data for GLP-related work. A writeable optical CD will permit this. We will also need additional computing power in the form of a Power PC100. By the second year we also anticipate doing larger numbers of posterior segment surgical models and will need mechanized vitrectomy capabilities. The Storz representative will make such an instrument available sporadically during the first year, but we will need more reliable access to such a machine as we progress further into the study.

Supplies. These are for immunoassays.

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a. Introduction

1. Objective

The overall objective of this proposal is to evaluate the efficacy of an antibody (LM609) to the integrin $\alpha_v\beta_3$ in the treatment of ocular neovascular disease. The initial goal is to test this reagent in inhibiting new blood vessel growth in an a rabbit corneal eye model. Once this system has been thoroughly characterized we will extend these studies to examine angiogenesis in the posterior segment with particular focus on inhibiting retinal neovascularization of the type seen in proliferative diabetic retinopathy. The ultimate goal is to enter phase one clinical trials with a humanized form of this antibody. During the course of the animal studies it should be possible to further elucidate the molecular mechanisms underlying angiogenesis and its inhibition by a reagent to a specific cell surface receptor now known to be important in regulating the angiogenic response to various environmental stimuli.

A better understanding of the molecular mechanisms underlying the proliferation of new blood vessels in diabetic eye disease, and neovascular eye disease in general, would provide a rationale basis for the development of therapeutic reagents. While such reagents would not necessarily cure the underlying disease, they could be useful in ameliorating the clinically significant pathological changes observed in these conditions. Based on the studies discussed in Preliminary Results, LM609 may represent such a reagent. By studying its effect in an eye model of diabetic and non-diabetic eye disease we may gain a better understanding of the events involved in vasoproliferative disorders in general.

The approach we have taken to inhibiting ocular angiogenesis is a novel one. Previous work has been directed at identifying the angiogenic molecules that may play a role in ocular neovascularization. This has formed the basis for therapeutics that involve the use of inhibitors directed at specific stimulatory molecules, or at angiogenesis in general (without a defined mechanism). Without a defined mechanism, it is very difficult to develop antagonists with more desireable therapeutic properties (e.g., higher therapeutic index, less side effects, increased specificity). Inhibitors directed at a specific stimulator (e.g., an antibody to VEGF or FGF) may be completely ineffective, or only partially effective, if multiple factors can serve as stimuli; suppression of one particular stimulus may result in upregulation of alternate angigogenic substances. The use of integrin antagonists represents a potentially powerful therapeutic; the mechanism of action is well characterized, it appears to be highly specific with no detectable effect on mature blood vessels, and, most importantly, appears to interfere with angiogenesis at a final common pathway in the neovascular process irregardless of the stimulus. In order to proceed to phase I clinical trials, a rigorous series of preclinical testing is necessary and must conform to Good Laboratory Practice (GLP) standards. The funds requested in this application are to purchase equipment and hire personnel specifically for the purposes of completing the necessary preclinical toxicology, pharmacokinetic and bioassay experiments.

2. Rationale: Background and Current Status of Research in the Field of Angiogenesis and Neovascular Eye Disease

Death from diabetic coma dropped dramatically with the introduction of insulin in the 1930's. Once the life expectancy of diabetics was extended with this medication, the focus of clinical investigations in diabetes shifted to the microvascular abnormalities that are the hallmark of the disease. No where in the body are these pathological changes associated with greater morbidity than in the eye. Maintenance of well-defined avascular regions of the eye are essential for optical transparency and vision; once new

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Quarterly Report to BAT ONC

Statu

Project:	**EMD 121 974 Cyclic Peptide**	**Account No.:**	**62 041**
Indication:	**Palliative treatment of solid lung, colorectal and breast cancer**	**Phase:**	**preclinical**
Project leader:	**Dr. Gabriele Noll**	**Signature:**	

Milestone Tracking: *MS 3 first patient in (Phase I)* **Target Date:** *last report* *current* **01.03.9**

Deviation:

Comment
Replacement of EMD 85 189 by EMD 121 974 (inner salt) has taken place

Status and Progress (since last report to BAT)

CMC (Chemistry, Manufacturing, Control)
The synthesis in solution is used at ZVE for 3 kg material. The amount is sufficient to cover toxicological and clinical trials until May 1998.
Further 20 kg from ZVE are expected in May 1998.
In addition 5 kg compound has been ordered from Orpegen for next year.

Preclinical
No negative results have been found in experiments of side reaction pharmacology.
In vitro pharmacology studies and in vivo experiments in chick CAM model and SCID mouse human skin transplant tumor model have been finished with positive results.
Nude mouse tumor model experiments are ongoing.
Further experiments in hamster dorsal window models are scheduled for 1998.
Genotoxicity (AMES-Test) and acute toxicity study have been finished.
Dose finding study for repeated dose toxicity in rodent was started in August 1997 and is ongoing. The repeated dose toxicity in rodent (4 week treatment) will start in September 1997.
Pharmacokinetics and toxicokinetics are scheduled for parallel development.
Development and manufacturing of a parenteral formulation and short term stability testing for the toxicological studies will be finished in September 1997.

Clinical
CDP 1 is scheduled to be finished during September 1997.
Discussion of CDP 1 with external experts and authorities is scheduled for 4th quarter 1997.
Preparation of Phase I studies started at the beginning of July 1997.

Marketing/Licensing
The first marketing / REG strategy meeting was held in August 1997.

Regulatory Status
The general authority concept and strategy was prepared at a meeting in June 1997.

Miscellaneous

Key Issues [redacted]

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Key Decisions
BAT approval to start phase I trials is due for December 1997.

Attachments

Routine **For Decision Making**
Project Schedule

EMD 121 974 Cyclic Peptide: Project Schedule

MERCK

	Task	Durat.	Start	Finish
3	MS 2 first treatment day tox	0w	01.09.97	01.09.97
4	MS 3 first subject in (phase I)	0w	01.02.98	01.02.98
4	20 kg GMP synthesis	42w	11.08.97	25.05.98
5	5 kg external synthesis (Orpegen)	48w	01.10.97	01.09.98
3	pharmaceutical development	48,8w	03.02.97	18.12.97
6	formulation tox pH7	7,2w	14.07.97	01.09.97
7	formulation phase I	18w	23.06.97	19.10.97
0	medication phase I	10w	19.10.97	18.12.97
1	pharmacology	74w	01.04.97	31.08.98
9	animal pharmacokinetics	63,8w	02.06.97	19.08.98
9	toxycology	136,2w	30.04.97	01.12.99
0	metagenicity	26w	30.04.97	14.10.97
1	genotoxicity: mouse/typhone-test Lv.	26w	30.04.97	14.10.97
2	4wk-tox / mouse (repeated dose) RCC	22,7w	03.09.97	09.02.98
7	4wk-tox / cynomolgus (repeated dose)	33w	01.01.98	19.08.98
3	3month-tox / rodent	39w	01.04.98	01.12.98
8	3month-tox / no-rodent	39w	01.04.98	01.12.98
6	Clinical Development	287,1w	18.11.96	28.07.02
8	Clinical Team Selected	0w	25.03.97	25.03.97
0	CDP Preparation	129,1w	25.03.97	24.11.99
2	Approve CDP-1	0w	04.09.97	04.09.97
4	Approve CDP-2	0w	25.03.98	25.03.98
7	Phase 1 Core	113,3w	10.07.97	10.08.99
9	Start Phase 1 (Decision)	0w	02.01.98	02.01.98
0	FSI-Core 1 (Allocated to Test Drug) (MS 3)	0w	02.02.98	02.02.98
1	Phase I: NL (study 001)	75,7w	10.07.97	12.01.99
8	Phase I: NL follow up (stud. 002)	113,3w	10.07.97	10.08.99
4	regulatory affairs	238,8w	04.04.97	01.12.03
8	dossier	36,8w	16.07.97	20.03.98
8	Pre-BID package: USA	16,8w	16.07.97	08.11.97
2	BID: USA	34w	25.07.97	20.03.98

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Project EMD 121 974

International Development Plan (IDP)

Version 0.1

as of April 01, 1998

Project Manager: Dr. U. Grimm.....

approved by BAT-Head: Prof. Dr. Schnurr.....

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Project Summary

Project	EMD 121 974 / oncology	Account No.	62041
Indication	Palliative treatment of colorectal, breast and NSCL cancer	Phase	Preclinic
Project Manager	Dr. U. Grimm		
Last IDP review	April 1, 1998	Next scheduled IDP review	to be determined

Project objectives

Overall project objective is to develop within 7 years a product for the choosen markets in the above mentioned indications with clinical efficacy superior to established therapeutic regimes due to a new mechanism of action.

Objective of this development plan is to assess the development and marketing strategy including cost and resources.

Responsible: EPG

Proposed labelling

Responsible: Dr. Dr. Rohmann

Earliest:	Europe (EU)	Europe (non-EU ") CH	USA	Japan	SEA	Latin America	Rest of the World
Submission	2003	2003	2003	X	X	X	X
Launch	2004	2004	2005	X	X	X	X
Patent expiry	2016	2016	2016	2016	2016	2016	2016

X to be determined

Budget (assuming sole development)

mio DM	1997 actual	1998 estimated	1999 estimated	2000 estimated	2001 estimated	2002 estimated	2003 estimated	2004 estimated
External costs	1.0	7.8	8.2	14.3	17.0	8.5	4.8	4.7
Internal costs	2.3	4.0	3.3	3.0	3.1	3.4	2.4	2.1
Total	3.3	11.8	11.5	17.3	20.1	11.9	7.2	6.8

Total development costs:	89.7	Remaining development costs:	86.4
external:	68.4	external:	67.4
internal:	21.3	internal:	19.0
Proof of efficacy: Remaining costs:	60.5	Proof of efficacy: Target Date	1.9.2001
external:	47.1		
internal:	13.4		

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Key success factors	How measured
• Efficacy profile: effective in treatment refractory patients due to new mechanism of action.	• Inhibition of tumor growth
• Long term efficacy due to lack of drug resistance	
• Tolerability profile: moderate toxicity	
• Dose regimen: solution for short term infusion	• I. v. formulation available

Development Strategy

- **Overall development plan**

Phase I
Selected doses: 30 mg/m² (starting dose); 50 % Cmax; 100 % Cmax; 150 % Cmax; 200 % Cmax;
Schedule: twice weekly infusion over 1h.
Phase IIa
Schedule dependent on Phase I pharmacokinetic data; supportive data for development.
Phase IIb
- pivotal studies for accelerated regulatory approval
- primary target variable Colorectal Ca. and NSCLC: Response to Therapy; Breast Ca.: Improvement in Quality of Life
- secondary target variables: Overall Survival, Disease-free Survival (Breast Ca.: Response to Therapy)
Phase III
Survival study in patients with NSCLC; Design: 40 % increase in median survival

Production of pre pivotal intermediate in 2 places externally, final GMP product in house.
Formulation for short term i.v. infusion has been developed.
Pharmacology
Additional nonclinical data for refined dosing and scheduling from mouse tumor model are ongoing.

Cooperation with NCI on Kaposi's Sarcoma and brain tumors.

Responsible: all EPG members

- **Next steps**

Phase I
- Start 3/98: 3 patients with single administration to determine pharmacokinetic parameters
- Determination on final scheduling after results of initial 3 patients
- Start CDP 2 update 3/98

Toxicology
- 4-week-toxicity-study in the cynomolgus monkey
- appropriate chronic toxicity studies in 2 species (monkey and rodent)

Production
Quality control of pre pivotal intermediate from Sygena and Orpegen. Final GMP synthesis of drug substance. Preparation of medication for chronic toxicity studies and patients in phase I and II.

External cooperation
Finalize contract with NCI.

Responsible: all EPG members

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- **Preclinical development plan**

Pharmacology

Status
EMD 121 974 is a potent and selective alpha v integrin inhibitor reducing tumor growth by blocking tumor induced angiogenesis through induction of apoptosis of cytokine activated endothelial cells.
Potency and selectivity was shown in vitro on cellular and sub-cellular level.
In vivo antiangiogenic activity was shown in cytokine or tumor mass induced angiogenesis in chicken CAM model, in mouse retinal vascularization model and corneal neovascularization model in rabbit.
Tumor growth reducing activity was shown in chicken CAM tumor growth models, s.c. melanoma model in nude mice, and in i.d. melanoma model in SCID mouse / human skin transplant model.

Plan
Refined dosing and scheduling in mouse tumor model is ongoing at TSRI.
Positive initial results in spontaneous pancreatic tumor model at UCSF are to be repeated.
Positive initial results in dorsal window chamber model with melanoma in hamster at Munich are to be repeated.

Pharmacokinetics and metabolism

Status
ADME studies in mice and Cynomolgus monkeys were performed after single iv and additionally in mice after single ip administration of unlabelled or ^{14}C-labelled EMD 121 974.

Plan

All toxicological studies are accompanied by toxicokinetic studies using a specific LC-MS/MS method for the determination of parent drug.

Toxicology

Status:

Genotoxicity:
- in vitro: Ames test, Mouse-Lymphoma-Test

Acute Toxicity:
mouse, i. v.

Repeated dose toxicity:
- EMD 85 189, 14 day pilot toxicity, mouse, i.p.
- EMD 121 974, 7 day orientating toxicity, mouse, i.v.
- EMD 121 974, 4 week, mouse i.v.

Plan:

Genotoxicity:
- in vivo: test for chromosomal damage with mouse hematopoietic cells

Repeated dose toxicity:
- EMD 121 974, 4 week, Cynomolgus monkey, i.v.
- EMD 121 974, subchronic, mouse, i.p.
- EMD 121 974, subchronic, Cynomolgus monkey, i.v.

Responsible: Drs. Goodman, Haunschild, Jonczyk, Rösener

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- **Chemistry, Manufacturing, Control (CMC)**

Drug substance

- costs per kg in 1996: 398 TDM
- costs per kg in 1999: 87 TDM
- costs per kg in 2000: 30 TDM

The API (active pharmaceutical ingredient) is produced in a 12 step synthesis. At present only steps 9 (key intermediate) to 12 (final product) are conducted by Merck. The production of step 8 (pre pivotal) has been outsourced to two different companies for different approaches (one solid and one solution phase synthesis). Major efforts are taken in ZVE in order to optimize the current synthetic route for a better yield and a reduction in costs. It seems to be reasonable that the costs will be reduced by 65 % (from 87 TDM to 30 TDM) over the next three years.

Production for expected cooperation with NCI ongoing: Orpegen delivery of 20 kg step 8 expected in May 98, yield should be 5 kg of EMD 121 974.

Sygena delivered 4.5 kg of pre pivotal intermediate. Sygena follow up deliveries are expected to be 16.5 kg until July 1998.
Synthetic steps for final GMP product will be in house continuously.

Monograph: Validation of analytical methods initiated
Stability: One batch in long-term stability program
IND: Search for a suitable external consultant for review of the respective IND-documents (in progress)

Drug product

Strength under development:
45 mg/30 ml, other volumes are possible

Manufacturer:
Lipha, Semoy

Stability data:
Long term stability testing has been started in March 98.

Development of an oral formulation is postponed.

Responsible: Drs. Arnold, Jonczyk, Krah, Kurz

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- **Clinical development plan**

Clinical target profile:	Current clinical status and profile:
Efficacy: • inhibition of tumor growth/reduction of non-resectable tumor load • improvement in QoL • increase in survival time • Palliation of tumor related symptoms	Efficacy: • not applicable
Safety: • no effect on postoperative wound healing, peptic ulcer healing • no severe effect on bone turnover • minor effects on macrophage phagocytosis and collateral development in coronary heart disease	Safety: • not applicable

Phase I: Open label 2 center study with pharmacokinetically guided dose escalation; Follow-up study for patients responding to therapy
Objectives: identification of safe dose range; Characterization of frequent side effects; Determination of pharmacokinetic parameters; Evaluation of efficacy (biological/clinical)
Starting dose: 1/10 th of LD10 (mg/sqm); Number of patients: 15 - 30; Duration: 28 days active treatment

Phase IIa: 3 open, uncontrolled, multicenter studies (Colorectal Ca., Breast Ca., NSCLC); 3 Follow-up studies for patients responding to therapy
Objectives: Determination of antitumor activity; Proof of concept; Characterization of side effects
Design: 4 different doses in parallel using 2-stage Simon Design; Number of patients: 440
Duration: 6 months active treatment

Phase IIb: 3 randomized, multinational studies (Colorectal Ca., Breast Ca., NSCLC); 3 Follow-up studies for patients responding to therapy
Objectives: Confirmation of efficacy; Characterization of side effects; Improvement in Quality of Life
Design: 3 parallel groups; Number of patients: 477
Duration: 6 months of active treatment

Phase III: Randomized, multinational study in NSCLC with 2 parallel groups comparing EMD 121 974 with standard 2nd line therapy
Objective: Proof survival benefit; Number of patients: 518
Duration: a maximum of 24 months active treatment

Responsible: Drs. Goddemeier, Kovar, Perschl

- **Regulatory strategy**

EU:
At the marketing/REG strategy meeting (August 21, 1997)it was decided to use the centralized procedure. A trade mark has to be available before the start of the procedure.
European non-EU countries:
Submission in Switzerland in parallel to EU. Other national submissions after having finalized EU procedure. Central East European countries require a Free Sales Certificate.
USA:
NDA submission in parallel to EU, together with an experienced partner.
Japan:
The strategy has to be discussed
Other countries (e.g. SEA, Latin America):
Submissions are planned in a second step, some countries require a Free Sales Certificate of the country of origin.

Responsible: Dr. Weiss

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- **Marketing / Profiling strategy**

Relevant market (mio DM): Breast Ca., Lung Ca., Colorectal Ca.

%	year / region	1997	2002
26.3	Europe**	1602	2814
42.7	USA	2602	4569
21.5	Japan	1310	2300
	total (world)	6093	10700

- *Cancer world market in 2002 (Source: Frost & Sullivan 1996)
  ** D, E, F, GB, I

NPV: 1,797 mio DM (best case)
Peak sales (5th year): 1,856 mio DM (best case)
Break-even point: 1 year after launch
Target price: 10,000 DM (ex factory) / treatment cycle of 6 month
Cost of goods: 18.9 %

Positioning statements
- Palliative treatment of CRC, Lung Ca., and Breast Ca.

Pricing (draft)
- Initial health economic consideration may allow for a pricing of 10 TDM for a 6 month treatment period with EMD 121974 given that the drug treatment prolongs life significantly and enhances quality of life.

International market penetration
- EU and USA should be approached in parallel as early as possible. Latin America and SEA will follow as soon as sanitary approval is given either in EU or USA. For Japan no marketing strategy is yet in place.

Health economics
- Preliminary review of secondary costs and outcomes data has been performed. This analysis leads to the hypothesis that the price for EMD 121974 can be justified. Nevertheless, concrete primary data on treatment patterns, patient management and costs in palliative care still have to be collected (1998 - 2000) in order to prove this hypothesis. These data will be country specific.

Competitive environment
- Thalidomide, ZD0101, Neovastat and Marimastat in phase II

Specific issues: major threats
- An orally available antiangiogenic compound will be launched prior to EMD or within the same time.
- Marketing approval for NSCLC cannot be achieved.
- Pricing levels for palliative treatment become eroded by an earlier introduction of the quasi-generic thalidomide into the market.

Trade mark
- world wide branding will start in the 4th q. of 1998. Brand Institute, NY)

Responsible: Dr. Dr. Rohmann, Fr. Paschen

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Key dates (Responsible: Dr. Grimm)

Decision Points	Initial schedule	Current schedule	Achieved
DP 1 (Project start)			15.11.1996
DP 2 (First subject in)	18.12.1997	26.02.1998	26.02.1998
MS 3 B (First patient in phase II b)			
DP 3 (Start phase III)			
DP 4 (NDA submission)			
DP 5 (Product launch)			
Milestones			
MS 1 (start of first GLP toxicity study)			1.09.1997
MS 2 (First subject in phase I allocated to test drug)	1.02.1998	27.04.1998	
MS 3 (First patient in - allocated to test drug)			
MS 3A (First patient in phase II a)	1.01.1999	1.07.1999*	
MS 3B (First patient in phase II b)	1.11.1999	1.06.2000	
MS 3C (First patient in phase III)		1.09.2001	
MS 4A (Last patient out phase II b allocated to test drug)		1.06.2001	
MS 4B (Last patient out phase III - allocated to test drug)		1.08.2004	
MS 5 (First NDA submission)	1.02.2003		
MS 6 (First product launch)	1.02.2004		

* Cause of delay: Lack of raw material caused delay in toxicity studies
Delay in phase I

Specific issues

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Go / No go criteria
• low tolerability (intolerable adverse events) • lack of efficacy in patients • unacceptable dosage regimen • orally available competitor product with similar efficacy and side effects targeting the same receptor successful in pivotal clinical trials • orally available competitor product with comparable efficacy and comparable side effects targeting other receptors successful in pivotal clinical trials Responsible: all EPG members
Anticipated obstacles / issues
• chronic treatment • possible interference with collateral formation in coronary heart disease, wound healing, ulcer healing and bone turnover • difficult synthesis • high price of the compound • high dosage • question, whether QoL is a suitable parameter in phase II b pivotal studies in order to get a marketing authorisation Assumptions: • Production of the compound at costs of 20,000 DM/kg will be possible. • First on the market (Europe and USA). Responsible: all EPG members
Contingency plans for high-impact obstacles
• Development of EMD 121 974 in the indications cancer of the pancreas or of the kidney as fast track. Responsible: all EPG members

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Project Organisation

Steering Committee (BAT ONC)	Prof. Erhard Schnurr Prof. Siegfried Matzku Dr. Dr. Sven Rohmann Dr. Cornelius Sobel Dr. Lothar Finke Dr. Jürgen Uhl Dr. Birgit Möllers Dr. Paul Mendelsohn
Project Manager	Dr. U. Grimm
Project Assistant	Ms. M. Bruntke
Project Team	
Preclinical	Dr. Simon Goodman Dr. Alfred Jonczyk Dr. Jutta Haunschild Dr. Sigrid Rüegner
CMC	Dr. Markus Arnold Dr. Alfred Jonczyk Dr. Mirko Krsh Dr. Thekla Kurz
Clinical	Dr. Andreas Kovar Dr. Astrid Perschl
Biostatistics	Hr. Thomas Goddemeier
Regulatory Affairs	Dr. Carl-Martin Weise
Marketing Health Economics	Dr. Dr. Sven Rohmann Ms. Britta Paschen

External Partners

Pharmacology	TSRL USA
CRO phase I	Quintiles
Clinical development in Kaposi's Sarcoma and brain cancer	NCI, USA

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Memo

An: Erhard Schnurr/EMD/Merck
Kopie: (Blindkopie: CN = Tilo Netzer/OU = EMD/O = Merck)
Blindkopie:
Von: Lothar Finke/EMD/Merck
Datum: 03.02.97 12:34:22
Thema: Rechtsstreit Scripps

Sehr geehrter Herr Prof. Schnurr,

von der Rechtsabteilung wurde mir am Freitag letzter Woche ein Schreiben vorgelegt, in dem wir aufgefordert werden bis zum 07. Februar einen Entwicklungsplan für EMD 85189 vorzulegen. Das EPG Core Team hatte hierfür, da wesentliche Daten aus Toxikologie und Pharmakologie fehlen, September / Oktober 1997 projektiert. Ich halte es daher für absolut unmöglich die Zeitvorstellung der Rechtsabteilung einzuhalten.

Mir ist selbstredend klar, daß es dem Verfahrensablauf wesentlich helfen würde, wenn wir diese Dokumente hätten. Dennoch möchte ich davor warnen jetzt einen Schnellschuß abzugeben, gerade weil diese Unterlagen dann zum Gegenstand eines Rechtsstreites werden. Bevor wir solche Dokumente aus dem Haus geben, müssen diese fundiert recherchiert und ausgearbeitet worden sein. Es kann nicht sein, daß wir ein Draft abliefern, welches intern allenfalls den Wert einer Absichtserklärung hat, extern aber zur Argumentationsführung vor einem Gericht herangezogen wird. Da machen wir uns doch unglaubwürdig!

Ich hoffe, daß Sie diese Auffassung teilen und entsprechend kommunizieren.

Mit freundlichen Grüßen

L. Finke

Erstellt am:03.02.97 12:34:22 von:Lothar Finke

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PLAINTIFF'S EXHIBIT 468

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TRANSLATION

Memorandum

To: Erhard Schnurr/EMD/Merck

Copy: (CC: CN = Tilo Netzer/OU = EMD/O = Merck)

CC:

From: Lothar Finke/EMD/Merck

Date: Feb. 3, 97 12:34:22

Subject: Litigation Scripps

Dear Professor Schnurr,

The legal department presented a letter to me on Friday of last week, requesting us to provide a development schedule for EMD 85189 by February 7th. The EPG Core Team had a projected September/October 1997 for this since crucial data from toxicology and pharmacology was missing. I therefore feel that it is absolutely impossible to comply with the time frame of the legal department.

I personally feel that it would be advantageous in the process development if we had these documents. However I would like to caution to provide a quick decision since these documents would then become an issue in the case of a litigation. Prior to releasing such documents, they have to be well founded, examined and completed. A draft version that may only have the value of an internal declaration of intention, may not be turned over, and since it may be used externally as an instrument of argumentation by a court. We will only look untrustworthy!

I hope that you share this opinion and communicate accordingly.

With kindest regards,

L. Finke

Issued: Feb. 3, 97 12:34:22 by: Lothar Finke

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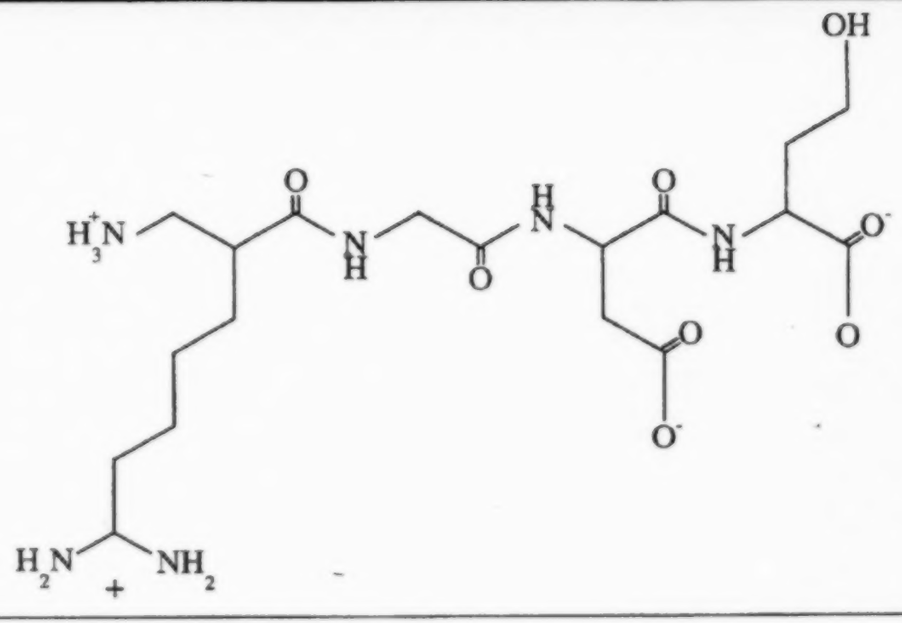
RGDS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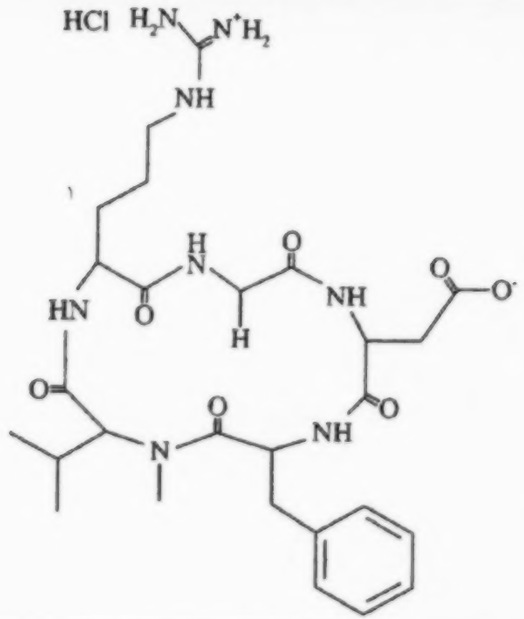

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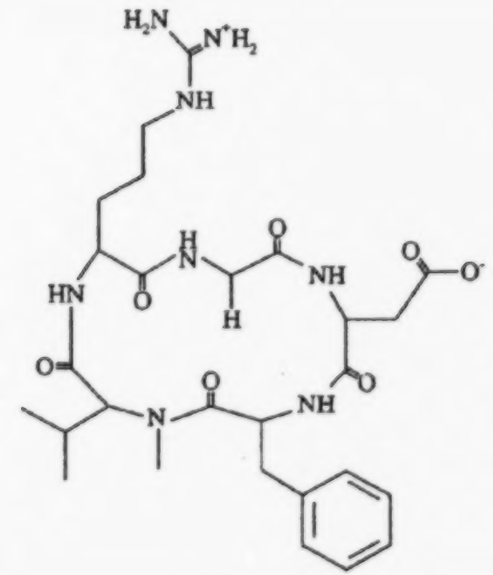
cRGDf-NMeV-inner salt



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